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Glomerular binding of anti-dsDNA autoantibodies: The dispute resolved?

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The binding of anti-double-stranded DNA (anti-dsDNA) autoantibodies to the glomerular basement membrane (GBM) in lupus nephritis can be explained by two mechanisms: (1) direct crossreactive binding to intrinsic glomerular antigens; (2) nucleosome-mediated binding to heparan sulfate in the GBM. Kalaaji *et al.* demonstrated using novel techniques that glomerular *in vivo*-bound antoantibodies bind to nucleosomes/dsDNA derived from apoptotic cells and not to intrinsic glomerular structures.

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Background

In general, lupus nephritis is associated with high titers of circulating high-affinity, IgG anti-double-stranded DNA (anti-dsDNA) antibodies and glomerular immunoglobulin deposits. Furthermore, elution of immunoglobulin from glomeruli revealed enrichment for anti-dsDNA antibodies. Therefore, it has been postulated that anti-dsDNA autoantibodies are nephritogenic in lupus nephritis. Later it became clear that not dsDNA itself but the nucleosome is the major driving autoantigen leading to the formation of nucleosome-specific antibodies, followed by the appearance of anti-dsDNA and antihistone antibodies as a result of epitope spreading.[1] In systemic lupus erythematosus patients and lupus mice, nucleosomes can be found in the circulation, which are spilled from apoptotic cells as a result of a defective removal of apoptotic cells, due to either an enhanced apoptosis or a defective cleaning machinery.[2] The nucleosome is composed of 146 bp of dsDNA, wrapped twice around an octamer of histones (pairs of the histones H2A, H2B, H3, and H4), and contains both negatively charged domains (that is, dsDNA) and positively charged domains (that is, histones).

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Crossreactivity of anti-dsDNA antibodies with glomerular antigens

The indirect evidence for the relation between anti-dsDNA antibodies and lupus nephritis has fueled experimental work to demonstrate more directly the nephritogenicity of anti-dsDNA antibodies. As lupus nephritis is regarded as a prototype of an immune complex-mediated disease, reconstituted dsDNA/anti-dsDNA complexes were injected into animals. However, this never led to a glomerular localization of these complexes. In contrast, injection of anti-dsDNA antibodies alone did sometimes lead to glomerular localization, which was used as an argument for the direct nephritogenicity of anti-dsDNA antibodies. In the early 1980s, seminal papers were published describing the broad reactivity of lupus-derived monoclonal anti-dsDNA antibodies that promiscuously recognized a great variety of non-related (glomerular) antigens.[3]

Several studies in mice, in which anti-dsDNA antibodies were injected or anti-dsDNA hybridomas were inoculated, showed evidence for glomerular binding of anti-dsDNA antibodies.[4] Immunoglobulin isolated from serum of lupus patients bound to isolated glomeruli. This could be inhibited by dsDNA but was not abrogated by DNase I treatment of the glomeruli.[5] Together with the finding that DNase I treatment did not abolish the binding of anti-dsDNA antibodies to glomerular antigens, this led to the formulation of the hypothesis that sub-populations of anti-dsDNA antibodies could bind directly to intrinsic glomerular antigens, such as laminin, heparan sulfate (HS), and α-actinin (reviewed by Waldman and Madaio[4]). It appeared that broadly crossreactive anti-dsDNA antibodies were the most nephritogenic, lending further support for the crossreactive recognition of glomerular antigens. The crossreactivity of anti-dsDNA antibodies to non-nucleosomal antigens was explained to be due to (1) the negative charge, as in HS linked to proteoglycans; (2) common structures, such as phosphodiester groups in phospholipids; and (3) molecular mimicry of dsDNA structures, as has been suggested for α-actinin.

Pathogenic anti-dsDNA antibodies have been reported to react with α-actinin. Together with the observation that sera of systemic lupus erythematosus patients and lupus mice have high titers of anti-α-actinin antibodies,[6] these data seem to support the crossreactivity hypothesis. But the logical prerequisite for this crossreactivity model is that these antigens are accessible on the surface of glomerular cells or in the glomerular basement membrane (GBM). There is no clear evidence that the podocytic protein α-actinin is expressed at the cell surface or extracellularly, in contrast to the other crossreactive targets. These studies indicate that some, but not all, anti-dsDNA antibodies can bind to glomerular antigens and to the GBM *in vitro* and *in vivo*.

Nucleosome-mediated autoantibody binding to the glomerular basement membrane

It was found that anti-dsDNA antibodies and immunoglobulin eluted from kidneys from systemic lupus erythematosus mice and patients could bind to the negatively

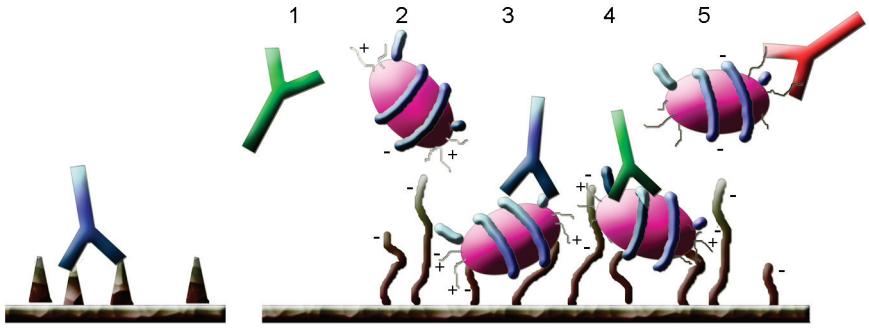

Figure 1 | Schematic representation of the two hypotheses for the glomerular binding of autoantibodies in lupus nephritis. From left to right: direct binding of crossreactive autoantibodies to non-nucleosomal glomerular antigens; nucleosome-mediated binding of complexed autoantibodies to heparan sulfate (HS). The nucleosome-mediated binding does not occur with (1) non-complexed anti-nuclear antibodies or (2) free nucleosomes. Binding of (3) anti-dsDNA (blue) or (4) anti-nucleosome antibodies (green) will decrease the density of negative charges of the nucleosome. This will enhance binding of the complex to the negatively charged HS and lead to nucleosome-mediated binding. In contrast to this, binding of (5) anti-histone antibodies (red) to the nucleosome will decrease the amount of positive charges, which reduces the capacity to bind to HS in the glomerular basement membrane.

charged HS side chain of HS proteoglycans.[2] It was hypothesized that this reactivity with HS was due to crossreactivity of anti-dsDNA antibodies with the anionic sites of HS mimicking dsDNA. However, after extensive purification of anti-dsDNA antibodies, the binding to HS was completely lost and could be restored only by the addition of histones and dsDNA.[2] More detailed analysis showed subsequently that DNase I treatment of complexed antibodies did not remove dsDNA completely, whereas histones remain bound. More vigorous purification methods including DNase I treatment followed by stringent high-salt conditions were required to remove all nucleosomal material.[7] Many of the observations on crossreactivity were based on the use of monoclonal antibodies. These antibodies become complexed to nucleosomal material released from dying hybridoma cells. This gives rise to spurious binding properties, which are not abrogated with DNase I treatment.

To evaluate whether binding to HS could also occur *in vivo*, renal perfusion studies were performed. Indeed, anti-nucleosome/anti-dsDNA autoantibodies complexed to nucleosomes bound strongly to the GBM, in contrast to purified non-complexed autoantibodies. Removal of HS before the perfusion of the nucleosome-complexed antibodies prevented glomerular binding.[7] This is supported by an almost complete absence of staining for HS in the GBM in both human and murine lupus nephritis. The disappearance of HS staining is due to masking of HS by nucleosome/immune complexes. Recently, we could show that elution of immune complexes from renal sections restored the staining of HS (unpublished data). Further support was the ability of heparin to prevent the glomerular binding of nucleosome-complexed autoantibodies and to postpone murine lupus nephritis *in vivo*.[2] Subsequently, we could show *in vivo* that glomerular binding especially occurred if nucleosomes were complexed to anti-dsDNA or anti-nucleosome and not to anti-histone antibodies.[2] The binding of anti-nucleosome or anti-dsDNA antibodies to the nucleosome will mask its negative charge and will facilitate the binding of the positively charged N-terminal histone tails to the negatively charged HS in the GBM. On the other hand, the binding of anti-histone antibodies to the N-terminal histone tails will mask their positive charge, preventing deposition of nucleosome/immune complexes (Figure 1).

Besides HS, collagen IV has also been proposed as a ligand for nucleosome-mediated binding to the GBM.[8] Treatment of the GBM with collagenase IV prevented binding, which could be restored only after the addition of collagen IV. It was suggested that nucleosomes bind to collagen IV via their histone parts, analogously to the situation with HS.[8]

Kalaaji *et al.*[9] (this issue) provide convincing evidence that nephritogenic autoantibodies bind to apoptotic chromatin structures in the GBM. In their previous study on murine lupus nephritis,[10] they already showed that there was no colocalization of *in vivo*-bound autoantibodies and antibody probes specific for α-actinin, laminin, or collagen IV. Unfortunately, they did not perform these analyses on the human kidney sections. The *in vivo*-bound autoantibodies in the GBM colocalized with chromatin identified with the TdT-mediated dUTP nick end labeling technique.[9,10] Although only a limited number of renal biopsies were evaluated, the impact of their current study[9] and their previous study[10] is considerable. Using elegant methods, they accurately define the glomerular localization and target antigens of anti-dsDNA autoantibodies in lupus nephritis. In our opinion, their findings should lead to a re-evaluation of the crossreactivity hypothesis.

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